2

Institute Report No. 291

Acute Dermal Toxicity of Diethyleneglycol Dinitrate in Rabbits

Larry D. Brown, DVM, LTC VC
and
Don W. Korte, Jr., PhD, MAJ. MSC

MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

September 1988

Toxicology Series: 155

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Acute Dermal Toxicity of Diethyleneglycol Dinitrate in Rabbits (Toxicology Series 155)--
Brown and Korte

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

Edwin S. Beatrice 20 Sept 88

Edwin S. Beatrice (date)
COL, MC
Commanding

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): Institute Report No. 291	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Mammalian Toxicology Branch, Division of Toxicology; 6b. OFFICE SYMBOL (If applicable): SGRD-ULE-T	7a. NAME OF MONITORING ORGANIZATION: US Army Biomedical Research and Development Laboratory
6c. ADDRESS (City, State, and ZIP Code): Letterman Army Institute of Research, Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21701-5010
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: US Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 62720A; PROJECT NO. 835; TASK NO. AB; WORK UNIT ACCESSION NO. DA 303913

11. TITLE (Include Security Classification)

Acute Dermal Toxicity of Diethyleneglycol Dinitrate in Rabbits

12. PERSONAL AUTHOR(S)

Larry D. Brown and Don W. Korte, Jr.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Institute	FROM 8/22/85 TO 10/1/85	1988 September	21

16. SUPPLEMENTARY NOTATION

Toxicology Series 155

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	acute dermal toxicity, rabbit, diethyleneglycol dinitrate / DEGDN, propellant, munition.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The acute dermal toxicity of diethyleneglycol dinitrate, DEGDN, was evaluated in male and female New Zealand White rabbits. Neat DEGDN was applied topically to the clipped dorsal skin surface under a semi-occlusive wrap for 24 hours. A limit dose of 2 g/kg did not produce deaths or clinical (systemic or dermal) signs, during the two-week observation period, that could be directly attributed to administration of the DEGDN. One intriguing observation was the occurrence of curly new hair growth on 6 of the 10 rabbits in the area where DEGDN had been applied.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Edwin S. Beatrice, COL, MS	22b. TELEPHONE (Include Area Code): 415-561-3600; 22c. OFFICE SYMBOL: SGRD-UL-Z

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE: UNCLASSIFIED

ABSTRACT

The acute dermal toxicity of diethyleneglycol dinitrate, DEGDN, was evaluated in male and female New Zealand White rabbits. Neat DEGDN was applied topically to the clipped dorsal skin surface under a semi-occlusive wrap for 24 hours. A limit dose of 2 g/kg did not produce deaths or clinical (systemic or dermal) signs, during the two-week observation period, that could be directly attributed to administration of the DEGDN. One intriguing observation was the occurrence of curly new hair growth on 6 of the 10 rabbits in the area where DEGDN had been applied.

KEY WORDS: Acute Dermal Toxicity, Diethyleneglycol Dinitrate, DEGDN, Rabbit, Propellant, Munition

PREFACE

TYPE REPORT: Acute Dermal Toxicity GLP Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-50
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NO.: 85003

STUDY DIRECTOR: Don W. Korte Jr., PhD, MAJ MSC

PRINCIPAL INVESTIGATOR: Larry D. Brown, DVM, LTC VC, Diplomate, American College of Veterinary Preventive Medicine and American Board of Toxicology

PATHOLOGIST: G. Tracy Makovec, DVM, MAJ VC, Diplomate, American College of Veterinary Pathologists

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Diethyleneglycol Dinitrate (DEGDN)

INCLUSIVE STUDY DATES: 22 Aug - 1 Oct 85

OBJECTIVE: The objective of this study was to evaluate the acute dermal toxicity of DEGDN in male and female New Zealand White rabbits.

ACKNOWLEDGMENTS

Gerald F.S. Hiatt, PhD, SSG James D. Justus, SPC James J. Fischer, SPC Scott L. Schwebe, Diane G. Arevalo, CPT Thomas N. Pool, DVM, and SPC Theresa L. Polk provided research assistance and animal care; SPC Paul B. Simboli assisted in the chemical preparation and analysis; Richard A. Spieler, Obie Goodrich, SFC Charles N. Farmer, and Charlotte L. Speckman provided animal care and facility management; Colleen S. Kamiyama, Dorothy Davis, and Julie Peacock provided secretarial assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 85003 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte Jr 15 Sep 88
DON W. KORTE JR, PhD / DATE
MAJ, MSC
Study Director

Barry D. Brown 26 Jul 88
LARRY D. BROWN, DVM / DATE
LTC, VC
Principal Investigator

Conrad R. Wheeler 15 Sep 88
CONRAD R. WHEELER, PhD / DATE
DAC
Chemist

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA (70-1n) 14 Sep 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85003

1. This is to certify that in relation to LAIR GLP Study 85003, the following inspections were made:

05 Mar 1985	- Protocol Review
17 Sep 1985	- Observation

2. The institute report entitled "Acute Dermal Toxicity of Diethyleneglycol Dinitrate in Rabbits," Toxicology Series 155, was audited on 13 August 1987.

Carolyn M. Lewis
CAROLYN M. LEWIS
Chief, Quality Assurance

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

Acute Dermal Toxicity of Diethyleneglycol Dinitrate (DEGDN) in Rabbits--Brown and Korte

INTRODUCTION

The Department of Defense is considering the use of either diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in new propellant formulations. However, considerable gaps in the toxicology data of the compounds were identified during a review of their health effects (1) conducted for the US Army Biomedical Research and Development Laboratory (USABRDL). Consequently, USABRDL has tasked the Division of Toxicology, Letterman Army Institute of Research (LAIR), to conduct an initial health effects evaluation of the proposed replacement nitrate esters. This initial evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP, includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, acute dermal toxicity in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

Objective of Study

The objective of this study was to determine the acute dermal toxicity of DEGDN in male and female New Zealand White rabbits.

MATERIALS

Test Substance

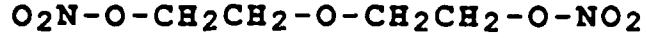
Chemical Name: Diethyleneglycol Dinitrate (DEGDN)

Chemical Abstracts Service Registry No.: 693-21-0

Code number: LAIR Code No. TA047

Physical State: Liquid

Molecular Structure:



Empirical formula: $C_4H_8N_2O_7$

Source: Hercules Incorporated
Radford Army Ammunition Plant
Radford, VA

Other test substance information is presented in Appendix A.

Vehicle

No vehicle was required as DEGDN is a liquid at room temperature.

Animal Data

Five male and five female young New Zealand White (NZW) rabbits (Elkhorn Rabbitry, Watsonville, CA, USDA #93A74) from a shipment that arrived at LAIR on 22 August 1985 were assigned to the study. One female rabbit (85F165) in the shipment was submitted for necropsy quality control 26 August 1985. The 10 rabbits were identified individually by ear tattoos. The animal weights ranged from 1940 to 2320 g on the day after receipt (23 August 1985) and from 2557 to 2715 g at dosing (16 September 1985). Additional animal data appear in Appendix B.

Husbandry

The rabbits were housed individually in stainless steel wire mesh cages in racks equipped with automatic flushing dumptanks. No bedding was used in any of the cages. Water was provided *ad libitum* by continuous drip from a central line. The diet consisted of approximately 150 g per day of Purina Certified Rabbit Chow No. 5322 (Ralston Purina Company, St Louis, MO, Lot Nos.: APR23852C, JUN04851C, and JUN04851B). The animal room temperature was maintained in a range from 18.8-24.4°C with a relative humidity range from 43 to 56% except for occasional spikes to 68% during room cleaning. The photoperiod was 12 hours of light per day.

METHODS

This study was performed in accordance with Environmental Protection Agency guidelines HG-Acute-Dermal, "Acute Exposure, Dermal Toxicity" (2), and LAIR Standard Operating Procedure OP-STX-30, "Acute Dermal Toxicity Study" (3).

Acclimation/Group Assignment

Study rabbits were quarantined by the Animal Resources Group (ARG), LAIR, for two weeks before being certified healthy by a staff veterinarian. During quarantine they were given sulfaquinoline (3.2 ml/326 ml water bottle *ad libitum*) for 7 days, for coccidial prophylaxis and one application of Canex® mineral oil (Pitman-Moore, Inc, Washington Crossing, NJ) into the ears for earmite prevention. After being certified healthy, the rabbits were transferred to the Toxicology Suite for the remainder of the study. The hair over the exposure site was initially clipped on 9 September 1985; however, because the rabbits were molting, dosing was delayed until 16 September 1985. The exposure site was clipped a second time on 15 September 1985, 24 hours before dosing.

Randomization for group assignment was unnecessary as there was only one dose level for each sex.

Dose Levels

A "limit test" was conducted in which 5 male and 5 female rabbits were assigned to a test group receiving 2.0 g/kg of DEGDN applied topically to the dorsum (skin over back).

Test Procedures

The application sites on the dorsal and lateral sections of the animals (surface area approximately 300 cm^2) were close-clipped with electric clippers (Oster® Model A5, Size 40 blade, Sunbeam Corp, Milwaukee, WI) 24 hours before applying the test compound. The animals were weighed, and the amount of compound required to provide the 2.0 g/kg limit dose was weighed. This quantity of the test compound (3.76-3.99 ml depending on the animal) was evenly distributed over the surface of an 8 x 8 in. piece of gauze dressing (Topper® Gauze Sponges, Johnson & Johnson Products, Inc, New Brunswick, NJ) which was then taped to the animal's back with hypoallergenic tape (Durapore® Surgical Tape, 3M Corp, St Paul, MN). The trunk of the animal was then wrapped with 4-in. Vetrap® bandaging tape (Animal Care Products, 3M Corp, St Paul, MN) to hold the compound in place and prevent the animal from ingesting the compound. The Vetrap® was anchored in place cranially and caudally by strips of Conform® elastic tape (Kendall Co, Hospital Products, Boston, MA). The patch and wrappings were left in place for 24 hours. No restraint of the animals was used except during the wrapping procedure.

When the wrappings and patch were removed the exposed area was gently wiped with a piece of saline-moistened gauze to remove any remaining test compound.

Observations

Clinical observations were recorded 3 times during the first 6 hours after dosing and daily for the remainder of the study. A second "walk through" observation was performed daily and only significant observations were recorded. The exposed area was examined and scored 1/2, 24, 48, and 72 hours after patch removal and then daily if lesions persisted. All lesions were noted and graded in accordance with Table 1.

TABLE 1: Evaluation of Skin Reactions

Formation	Score
Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate-to-severe erythema	3
Severe erythema (beet redness to slight eschar formation (injurious in depth)	4
Possible total erythema score	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (edges raised approx. 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Possible total edema score	4
Possible total score for primary irritation	8

Dermal lesions were evaluated with the unaided eye and recorded according to type, severity, and percent area exposed. Lesion type was classified as either erythema or edema, and severity was defined as slight, mild, moderate, or severe. Area categories were defined as less than 5%, 5 to 10%, 10 to 25%, 25 to 50%, and greater than 50% of the exposed area. Percent area exposed was determined by visual approximation. Body weights were recorded once a week during the course of the study.

Necropsy

All study animals were submitted for necropsy. Those which survived the 15-day study period were necropsied immediately after being given an overdose of sodium pentobarbital and sacrificed by exsanguination from severed axillary vessels. Skin was taken from the exposed area and examined microscopically.

Duration of Study

The study period was 15 days with a 14-day quarantine and a 10-day period to recover from molting. Historical study events are listed in Appendix C.

Changes/Deviations from Protocol

The study period was extended one day from 14 to 15 days to accommodate the necropsy schedule.

Raw Data and Final Report Storage

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Clinical Observations

Observations consisted of two major categories, systemic and dermal. No systemic signs clearly attributable to the compound were observed in any of the animals. The only systemic signs observed during the study were soft stools (diarrhea) in one male (85F158) and one female (85F166) on Day 15, a greenish-yellow nasal discharge in one male (85F159) on Days 9, 10, and 15, and curly new hair growth (where the compound had been applied) which became obvious on Day 15 on 6 of 10 rabbits (85F157, 85F158, 85F160, 85F164,

85F166, and 85F167). Abrasions were observed on the knees of all animals except 85F157 and were attributed to irritation from rubbing against the tape wrappings.

Slight erythema was observed initially in 3 of 10 rabbits after removal of wrappings (Table D-1 and Table D-2, Appendix D). This erythema persisted for 1-3 days.

A summary of the body weights during the quarantine and study period appears in Appendix E.

Gross Pathological Observations

There were no gross or microscopic findings in these rabbits at necropsy, following the 2-week observation period, that could be attributed to dermal exposure to DEGDN at the 2 g/kg dose level. A copy of the complete Pathology Report appears in Appendix F.

DISCUSSION

DEGDN produced no mortality in rabbits exposed to a limit dose of 2 g/kg. The systemic signs observed during the study were slight diarrhea in two rabbits, nasal discharge in one rabbit, and curly new hair growth in six rabbits. The diarrhea occurred at the very end of the study in both animals and was probably not related to the test compound. The greenish-yellow nasal discharge is characteristic of rabbit pasteurellosis (snuffles). The curly new hair growth is probably no more than an incidental finding, but it remains an intriguing observation.

Slight erythema was observed initially after removal of the wrappings in three of the 10 dosed rabbits. Two of the three rabbits had 10-25% of the exposed area affected, and the other rabbit had between 5-10% of the exposed area affected. The pattern of the erythema suggested that it may have been due to the wrapping procedure. The pathology report revealed no lesions attributable to the test compound. These minimal toxicological findings are consistent with the observation that significant quantities of test compound remained on the back after the 24-hr exposure. Dermal irritation studies with TEGDN and TMETN (4) support the findings in this study, i.e., DEGDN is non-irritating to skin. Acute oral toxicity studies with TEGDN and TMETN demonstrated the neuromuscular and central nervous system signs of toxicity typical of nitrate esters; however, no systemic toxicity was observed in the acute dermal toxicity studies with these compounds (4). As with TEGDN and TMETN,

none of the typical signs of nitrate ester toxicity were detected in this dermal toxicity study. The lack of systemic and dermal toxicity following dermal exposure to DEGDN suggests that there is little absorption of DEGDN by this route of administration.

CONCLUSION

A limit dose of 2 g/kg of neat diethyleneglycol dinitrate (DEGDN) was not toxic to rabbits following a 24-hr dermal exposure. DEGDN possesses a minimal potential for acute dermal toxicity.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846.

2. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Acute exposure, dermal toxicity. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.

3. Acute dermal toxicity study. LAIR Standard Operating Procedure OP-STX-30, Presidio of San Francisco, CA: Letterman Army Institute of Research, 18 May 1984.

4. Brown LD, Hiatt GFS, Morgan EW, Wheeler CR, Lewis CM, Johnson YC, Ryabik JRG, Okerberg CV, Makovec GT, Lollini LO, Mellick PW, Korte DW. Acute toxicity of TEGDN and TMETN liquid propellants. Laurel, MD: Chemical Propulsion Information Agency, 1985; CPIA Publication 436, pp. 313-320.

Appendix A: CHEMICAL DATA

Chemical name: Ethanol, 2,2'-oxybisdinitrate

Alternate chemical name: Diethyleneglycol dinitrate (DEGDN)

Chemical Abstracts Service Registry No.: 693-21-0

LAIR Code No.: TP047

Chemical structure:

$$O_2N-O-CH_2CH_2-O-CH_2CH_2-O-NO_2$$

Molecular formula: $C_4H_8N_2O_7$

Molecular weight: 196

Physical state: Pale yellow liquid

Density (g/cm^3): 1.38[1]

Analytical data: Refer to the attached data sheet, ARRCOM Form 213R. The compound chromatographed as a single peak (retention time 5.4 min) by HPLC analysis under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm); solvent system, 30% water, 70% acetonitrile; flow rate, 0.9 ml/min; detection wavelength, 205 nm.[2] NMR (300 MHz, CD_3CN): 3.75 δ (complex multiplet, 4H, $-CH_2-O-CH_2-$), 4.61 complex

---

[1] Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland; US Army Medical Bioengineering Research and Development Laboratory, 1983; DTIC No. ADA127846, p. 17.

[2] Wheeler CR. Toxicity Testing of Propell nts. Laboratory Notebook #85-12-023, p. 31. Letterman Army Institute of Research, Presidio of San Francisco, California.

Appendix A (cont.): CHEMICAL DATA

multiplet, 4H,-CH_2ONO_2).[3] Additional singlet signals of approximately equal intensity were observed at 2.08 d, and were due to sample impurities. Integration of all signals in the spectrum demonstrated that the sample contained 96.6% DEGDN. The impurities were not identified. IR(KBr): 2896, 1632, 1429, 1390, 1373,1279, 1139, 1032, 909, 857, 758, 707, 655, 572cm^{-1}.[4]

Stability: The DEGDN was shipped containing 18% acetone (a desensitizer) and arrived at LAIR on 12 December 1984. The acetone was removed by rotary evaporation prior to studies with the propellant. Analysis of the compound one year after it was received gave the results described above. Stability of the compound in corn oil (the dosing vehicle) was examined. As determined by HPLC, the concentration of DEGDN in corn oil emulsions 24 h after preparation was within 1% of the target value.[5]

Source: Radford Army Ammunition Plant, Radford, Virginia (prime contractor: Hercules Inc., Wilmington, Delaware).

Lot No.: RAD84M001S214

---

[3] Ibid. pp. 44-48.

[4] Ibid. pp. 49-50.

[5] Wheeler CR. Nitrocellulose - Nitroguanidine Projects. Laboratory Notebook #85-01-006, pp. 57-60. Letterman Army Institute of Research, Presidio of San Francisco, California.

Appendix A (cont): CHEMICAL DATA

DESCRIPTION SHEET FOR EXPLOSIVES, CHEMICALS, ETC

REPORTS CONTROL SYMBOL: EXEMPT-Para 7-2e AR 335-15 | PAGE 1 OF 1

TO:	FROM:	DATE: December 5, 1984
		MATERIAL: Diethylene Glycol Dinitrate (DEGDN)

MANUFACTURER: HERCULES INCORPORATED, RADFORD ARMY AMMUNITION PLANT
CONTRACT NO.: DAAA09-77-C-4007

SECTION A - DESCRIPTION OF LOTS

FROM NUMBER	THRU NUMBER	TOTAL NO. LOTS	TOTAL NET AMOUNT ACCEPTED
RAD84M001S214	-	1	5 lbs

PLACE MANUFACTURED: RADFORD ARMY AMMUNITION PLANT, RADFORD, VIRGINIA
SPECIFICATION AND AMENDMENT/DRAWING NO.: DOD-D-64015

SECTION B - DESCRIPTION OF MATERIAL

Requirements	Limit	Results
82.2°C Potassium Iodide Starch Paper Heat Test (KI)	10 minutes minimum	12
Nitrogen, %	14.10 minimum	14.15
Water, %	Info Only	0.43
Acidity	None	None
Alkalinity	None	None

REMARKS: DEGDN is desensitized with 15% or more of acetone for a total weight of 5 lbs, and packed in a DOT 6D 5 gallon drum with a DOT 2S liner, overpacked in a DOT-6J 30 gallon capacity drum with vermiculite as a cushioning agent around the 5 gallon drum and contained in the 30 gallon drum. Requested by shipping Order AMCCOM and COR letter SMCRA dated November 28, 1984 (DOT Exemption 5704).

SECTION C - CERTIFICATION

SAMPLING CONDUCTED BY: HERCULES INCORPORATED
TESTING CONDUCTED BY: HERCULES INCORPORATED

THE ABOVE MATERIAL COMPLIES WITH ALL SPECIFICATION REQUIREMENTS AND IS CERTIFIED TRUE AND CORRECT.

12-5-84 (DATE) — (SIGNATURE) — (TITLE)

THE ABOVE DESCRIBED LOTS ARE HEREBY ACCEPTED

Dec 6 1984 (DATE) — (TITLE) — FOR THE COMMANDER (SIGNATURE)

ARRCOM Form 213-R, 10 Aug 77 SEQUENCE NO. 374

Appendix B: ANIMAL DATA

Species: *Oryctolagus cuniculus*

Strain: New Zealand White (albino)

Source: Elkhorn Rabbitry
5265 Starr Way
Watsonville, CA 95076

Sex: Male and Female

Age: Young Adults

Animals in each group: 5 males and 5 females

Condition of animals at start of study: Normal

Body weight range at dosing: 2.56 - 2.72 kg

Identification procedures: Ear tattoo procedure (SOP OP-ARG -1), tattoo numbers 85F157-85F161, (males) and 85F164, 85F166-85F169 females (inclusive).

Pretest conditioning:

1. Quarantine from 22 Aug - 5 Sept 85

2. Animals were close-clipped and examined 24 hours before dosing

Justification: The laboratory rabbit is a proven mammalian model for dermal toxicity studies because of its size, ease of restraint, and skin permeability.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

Date	Event
22 Aug 85	Rabbits arrived at LAIR. They were checked for illness and quarantined.
22 Aug-5 Sep 85	Animals were observed daily.
26 Aug 85	Rabbits were tattooed. One female (85F165) was submitted for quality control necropsy.
5 Sep 85	Rabbits were removed from quarantine.
5-15 Sep 85	Rabbits were checked daily for illness.
9 Sep 85	Rabbits were clipped and examined. Dosing was delayed due to molting (purplish, erythematous skin).
15 Sep 85	Rabbits were reclipped.
16 Sep 85	Ten rabbits were dosed. Observations and clinical signs were recorded 3 times (1, 2, and 4 hours after dosing).
17 Sep 85	Wrappings were removed and rabbits were observed for dermal irritation and clinical signs of toxicity.
17-20 Sep 85	At 1/2, 24, 48, and 72 hours, dermal scoring was performed.

Appendix C (cont): HISTORICAL LISTING OF STUDY EVENTS

17-30 Sep 85	Rabbits were observed in the morning for clinical signs. A walk-through check was performed in the afternoon.
23,30 Aug; 6,10,16,23 Sep; 1 Oct 85	Rabbits were weighed once weekly.
1 Oct 85	Feed was withheld. Ten rabbits were weighed, observed, and then euthanized. Gross necropsies were performed. Skin from exposure sites was preserved for histological examination.

Appendix D: INDIVIDUAL DERMAL SIGNS--MALES

Table D-1

Animal Number	Dermal Signs	Duration of Dermal Signs (Days)	Severity*	Area†
85F157	Erythema	2-3	A	2
85F158	Erythema	2-3	A	3
85F159	None	N/A	N/A	N/A
85F160	None	N/A	N/A	N/A
85F161	Tape-site raw on Rt knee (not back)	1-2	A-B	1

* Severity Scores

A = Slight
B = Mild
C = Moderate
D = Severe

† Pertains to percent of exposed area exhibiting signs of dermal irritation. This value is determined by visual approximation.

1 = 5%
2 = 5 to 10%
3 = 10 to 25%
4 = 25 to 50%
5 = 50%

Appendix D (cont): INDIVIDUAL DERMAL SIGNS--FEMALES

Table D-2

Animal Number	Dermal Signs	Duration of Dermal Signs (Days)	Severity*	Area†
85F164	None	N/A	N/A	N/A
85F166	Erythema	1-2	A	3
85F167	None	N/A	N/A	N/A
85F168	None	N/A	N/A	N/A
85F169	None	N/A	N/A	1

* Severity Scores

A = Slight
B = Mild
C = Moderate
D = Severe

† Pertains to percent of exposed area exhibiting signs of dermal irritation. This value is determined by visual approximation.

1 = 5%
2 = 5 to 10%
3 = 10 to 25%
4 = 25 to 50%
5 = 50%

Appendix E: INDIVIDUAL BODY WEIGHTS (grams)

Animal Number	DAY OF STUDY						
	Q*1	Q8	Q15	Q19	0	7	15
				FEMALES			
85F157	2130	2140	2430	2545	2671	2830	2880
85F158	2320	2375	2516	2532	2557	2770	2737
85F159	2170	2210	2440	2626	2715	2799	2867
85F160	2195	2245	2450	2570	2617	2818	2775
85F161	2220	2240	2514	2583	2680	2756	2731
Mean	2707	2242	2470	2571	2648	2795	2798
± S.E.M	± 32	± 38	± 19	± 16	± 28	± 14	± 32
				MALES			
85F164	2170	2220	2443	2566	2689	2797	2843
85F166	2165	2165	2467	2530	2638	2784	2794
85F167	2165	2220	2452	2500	2653	2794	2877
85F168	1940	2060	2235	2485	2619	2727	2861
85F169	2250	2275	2453	2547	2668	2833	2835
Mean	2138	2188	2410	2526	2653	2787	2842
± S.E.M	± 52	± 36	± 44	± 15	± 12	± 17	± 14

* Q represents the quarantine period.

Appendix F: PATHOLOGY REPORT

Pathology Report
GLP Study 85003
Acute Dermal Toxicity (LD_{50}) Test

Test substance: Diethyleneglycol Dinitrate (DEGDN).

Investigator: MAJ Brown.

Species: Rabbit, NZW.

Age: Approximately 4 months old, 5 male, 5 female.

History: See LAIR SOP-OP-STX-30. All animals were killed by exsanguination following sodium pentobarbital anesthesia.

Gross Necropsy Findings:

PATH ACC#	ANIMAL ID#	SEX	DIAGNOSIS
38260	85F157	Male	Not remarkable (NR)
38261	85F158	Male	Purulent otitis media, bilateral
38262	85F159	Male	NR
38263	85F160	Male	NR
38264	85F161	Male	Purulent otitis media, left ear
38265	85F164	Female	NR
38266	85F166	Female	NR
38267	85F167	Female	NR
38268	85F168	Female	NR
38269	85F169	Female	NR

Appendix F (cont): PATHOLOGY REPORT

Pathology Report
GLP Study 85003

Microscopic Findings: Skin section control and treated.

PATH ACC#	DIAGNOSIS
38260 - 1 (control):	Not remarkable (NR)
38260 - 2 (treated):	NR
38261 - 1:	NR
38261 - 2:	NR
38262 - 1:	NR
38262 - 2:	NR
38263 - 1:	NR
38263 - 2:	NR
38264 - 1:	NR
38264 - 2:	NR
38265 - 1:	NR
38265 - 2:	NR
38266 - 1:	Dermatitis, heterophilic & histiocytic, subacute, multifocal, minimal.
38266 - 2:	NR
38267 - 1:	NR
38267 - 2:	NR
38268 - 1:	NR
38268 - 2:	NR
38269 - 1:	NR
38269 - 2:	Dermatitis, histiocytic & heterophilic, subacute, multifocal, minimal.

Comments: The gross lesions were considered incidental findings and not related to the treatment. No microscopic evidence of dermal toxicity was seen.

G. TRACY MAVOVEC, DVM
MAJ, VC
Comparative Pathology Branch

12 February 1986

Distribution List

Commander
US Army Biomedical Research and Development Laboratory (27)
ATTN: SGRD-UBZ-C
Fort Detrick, Frederick, MD 21701-5010

Defense Technical Information Center (DTIC) (2)
ATTN: DTIC-[illegible]A
Cameron Station
Alexandria, VA 22304-6145

US Army Medical Research and Development Command (2)
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21701-5012

Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

Chief
USAEHA Regional Division, West
Fitzsimmons AMC
Aurora, CO 80045

Chief
USAEHA Regional Division, North
Fort George G. Meade, MD 20755

Chief
USAEHA Regional Division, South
Bldg. 180
Fort McPherson, GA 30330

Commander
USA Health Services Command
ATTN: HSPA-P
Fort Sam Houston, TX 78234

Commandant
Academy of Health Sciences
United States Army
ATTN: Chief, Environmental Quality Branch
Preventive Medicine Division (HSHA-IPM)
Fort Sam Houston, TX 78234

Commander US Army Materiel Command
ATTN: AMSCG
5001 Eisenhower Avenue
Alexandria, VA 22333

Commander
US Army Environmental Hygiene Agency
ATTN: Librarian, HSDH-AD-L
Aberdeen Proving Ground, MD 21010

Dean
School of Medicine
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014

Commander
US Army Materiel Command
ATTN: AMCEN-A
5001 Eisenhower Avenue
Alexandria, VA 22333

HQDA
ATTN: DASG-PSP-E
Falls Church, VA 22041-3258

HQDA
ATTN: DAEN-RDM
20 Massachusetts, NW
Washington, D.C. 20314